A
Appendix: Practical Guide to Optical Alignment

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Lasers are often used for the alignment of an optical system, as they remain parallel over large distances. They can be specifically introduced into a setup for alignment needs or are sometimes already present for other purposes. Such a laser usually defines the optical axis, which should go through the precise center of all lenses and other optical elements.

A.1
How to Obtain a Widened Parallel Laser Beam

For alignment purposes and also for many microscopy applications, a widened parallel laser beam is needed. As a laser beam typically leaves the laser with a width of 0.2 to about 2 mm, we need to first enlarge its diameter and then make it exactly parallel. The enlargement is achieved by the use of a telescope, consisting of two lenses of different focal lengths adjusted such that the focal point of the first lens coincides with the focal point of the second lens. The laser beam will thus be focused to that focal point, where one can place a small (typically 20–100 μm) pinhole aperture. This pinhole serves to clean up the laser beam and remove unwanted distortions of the beam (Figure A.1). The expansion ratio of the laser is defined by the ratio of the two focal lengths. For microscopy application, one typically uses 1 inch optics to which the width of the widened laser beam can then be matched. To achieve optimal performance, one should use plano-convex lenses and orient the plane surface toward the focus. Minimizing the maximal angles over all optical surfaces is a general rule of thumb for optimizing performance.

It is difficult to make a widened laser beam exactly parallel, yielding a planar wavefront. Luckily there is a useful tool, which is available commercially, the *shear plate*.

The shear plate is a wedge of defined slope and thickness (Figure A.2), which is inserted into the beam path typically under 45°, with the slope of the wedge being oriented perpendicular to the beam. The laser reflects off the front and the back side, and both the reflected beams interfere with each other. With an incident parallel

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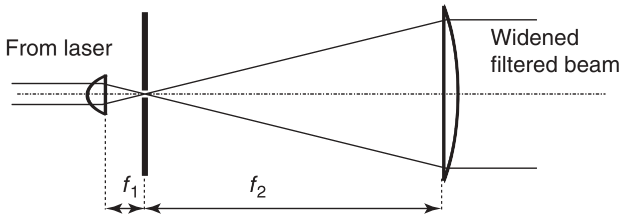

Figure A.1 A telescope with a pinhole in the focus for cleaning up the laser beam and making it a spatially extended parallel wave. Also note the direction of the plane and convex surfaces of the lenses to minimize the angles at the optical surfaces.

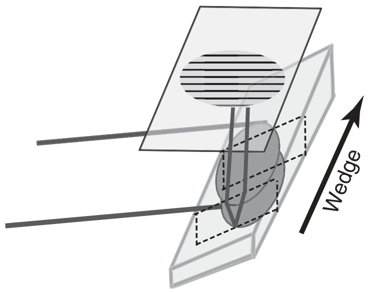

Figure A.2 A shear plate. Shown here is the principle of operation. A precision-manufactured glass wedge oriented sideways to the beam will lead to a series of parallel bright and dark stripes along the direction of propagation by the effect or light interference. Convergent of divergent light will lead to a tilted set of lines.

beam, the reflection from the front surface will thus generate one parallel beam, whereas the reflection from the wedged back surface will generate a parallel beam of slightly different orientation. Thus, a fringe pattern with straight lines will be visible, as can be seen from the interferences of the beams as shown in Figure A.2. The fringes are ideally oriented along the direction the laser beam was originally traveling. If the beam is converging or diverging, this fringe pattern will tilt sideways and changes its fringe spacing. Such a shear plate can be inserted in any parallel part of the beam as a useful tool for diagnosing errors in focusing or lens positioning.

If the lenses are passed under oblique angles or plano-convex lenses are inserted the wrong way around (see above), this can lead to higher-order aberrations, which are visible as curved fringes generated by the shear plate.

For the shear plate to work, we need two different paths (a difference of about 1 mm) to interfere, for which a laser with sufficient longitudinal and lateral coherence lengths is required. The coherence length of a light source describes the maximal optical path difference over which interference can be observed. Sometimes one also observes fringes with a contrast fluctuating over time. This

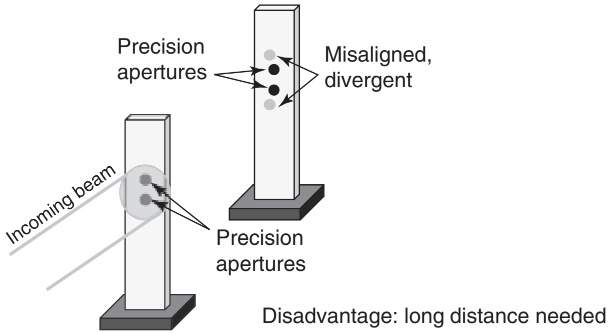

Figure A.3 An alternative way of determining whether the light is parallel using two sets of holes at predefined distances.

indicates that the longitudinal coherence length of the laser changes (possibly because of mode hopping).

Another useful tool is a set of precisely manufactured posts with two holes (1 mm diameter each), one drilled at nominal beam height and the other 3 mm above. This tool can be used to check the beam height above the table at various places in the optical setup. The two holes serve to define two beamlets, which should stay parallel over a large distance when illuminated by a single parallel beam. However, if the illuminating beam is converging or diverging, the distance between the two spots of light will change along the beam propagation (Figure A.3).

A.2 Mirror Alignment

Many optical setups contain mirrors to fold the beam path on the optical table. It is useful to have mirrors in a setup because they allow for an easy adjustment of the beam path. With two successive mirrors, one can do a *beam walk* and adjust position as well as angle of one part of the setup with respect to the next part. In a beam walk, the first mirror will have a larger influence on the position of any point downstream in the beam compared to the second mirror. The second mirror can be used to adjust predominantly the angle. Even though both the mirrors will affect both angle and positions, it is nevertheless useful to keep this predominance in mind: the first mirror affects mainly position; the second mirror, mainly angle. For example, to adjust the beam to pass through two pinholes, the first mirror should be used first to get the position right on the first pinhole and then the second mirror should be used to change the angle, to hit the second pinhole. Obviously, this beam walk process has to be repeated a number of times until the goal is achieved.

Mirrors are also quite useful when they can be flipped in and out of the beam path. This allows one to quickly and reproducibly switch between different setups, sharing some common component, for example, the laser or the microscope. Often magnetic base precision repositioning elements are more reproducible than magnetic flip mounts.

Despite these advantages, each mirror will also lead to losses. The losses are usually higher than the reflectivity specified in the catalog as it is hard to keep the mirror entirely free of dust. When dealing with setups that need to preserve the polarization of the light, the situation gets more difficult. Mirrors will almost always alter the polarization state when it is neither *p*- nor *s*-polarized. This is because they usually introduce a phase shift between *p*- and *s*-polarizations. To minimize this effect, one should aim for using the mirror as close to the perpendicular beam incidence as possible and prefer a metallic front surface mirror over a dielectrically coated mirror.

In fluorescence microscopy, one often uses a *dichromatic beam splitter* (sometimes also called *dichroic*). If one has complete freedom of design here, one should also try to use this at as close to normal incidence as possible because this will not only avoid polarization issues but also increase the steepness of the spectral cutoff characteristics. However, one has to be aware that this also shifts the design cutoff wavelength of the dichromatic beam splitter.

A.3
Lens Alignment

Optical systems often consist of multiple lenses. As a lens performs best for rays close to the optical axis (running through its center) and at small angles, all the individual lenses have to be well aligned with respect to an optical axis. This means that all the lenses have to be well centered with the optical axis being orthogonal to the lens surface at its center. As the centering of lenses is of utmost importance, the lenses are often placed in *XY* translational holders. To achieve the correct tilt of the lenses (usually modified without using special holders), the residual back reflection of the lens surface can often be used.

A.4
Autocollimation Telescope

A very useful tool is an *autocollimation telescope* comprising a grid, an illumination unit, and a camera. Autocollimators are used for the precise alignment of machine parts, are precision-manufactured, and thus quite expensive. With such a device rigidly mounted on the optical table to define the optical axis, one can quickly change between various focal planes and center each lens using a built-in crosshair target. By focussing on the cross-hair being reflected back from an optical surface, the tilt of that lens can be optimized. Ideally, the auto-collimated image is displayed on a little video screen. In this way the user can stand near the lens and align it while looking at the result of the auto-collimation on the screen (Figure A.4).

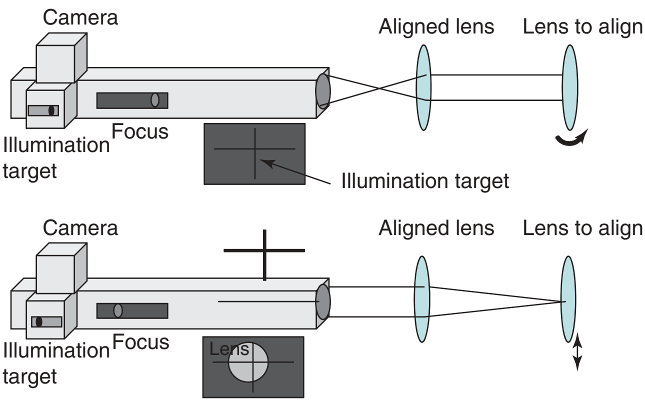

Figure A.4 The principle of operation of the autocollimation telescope. It is mounted in a fixed position to define the optic axis. Focusing on different optical surfaces allows the centering of the optical elements. Focusing on the illumination crosshair being reflected by optical surfaces helps precisely adjust their tilt to be perpendicular to the optical axis.

However, in many cases, one does not have access to an autocollimator and the alignment is done with a laser beam.

A.5
Aligning a Single Lens Using a Laser Beam

Starting with a parallel beam (e.g., checked for being exactly parallel using a shear plate, see above), shifting a lens sideways will move the focal point sideways by the same amount. If the illumination beam is significantly smaller than the lens size, the shift will also alter the beam direction, as the narrow laser beam then behaves almost like a ray as drawn in a ray diagram.

Steps to place a single lens into a widened parallel laser beam are:

1) Make sure that the laser beam is parallel.
2) Place an iris aperture A_1 (closed down to ∼1 mm) in the middle of the beam to define its center.
3) Place a second iris A_2 (closed down to ∼1 mm), where the focus of the lens should be. Adjust the iris A_2 such that the 1 mm beam goes through it, this defines the optical axis to which the lens should be aligned to.
4) Insert the lens such that the 1 mm beam generated by A_1 aims approximately at the center of the lens.
5) Fine-adjust the lens position by aiming the beamlet at the center of aperture A_2.
6) Adjust the lens tilt to make the lens perpendicular to the beam (see method below) and repeat steps 5 and 6 until satisfactory.

Tilting the lens from normal incidence should never be considered as a valid means of alignment of its focus. Always place the lens as perpendicular to the optical axis as possible and align its *XY* position. Then use the following trick to ensure that it is at normal incidence to the light beam.

A lens generates a small amount of reflected light from each of its surfaces. The light from the first surface is usually diverging (i.e., for biconvex lenses) and only useful for tilt alignment if the beam is small with respect to the lens size and the lens surface is not strongly curved. In this case, one should ensure that the reflected light returns through the first iris aperture A_1, defining the small center beamlet. One can also use the light returning from the second lens surface. This focuses at roughly half the focal distance before the lens (since it passes the lens twice). This focused spot can sometimes be easier to see and to use for the tilt adjustment. Also here, this spot should be in the middle of first aperture positioned at half the focal distance before the lens. It is useful to use a white business card with a ~1 mm hole punched into it, such that the back-reflected beam is visible when it misses this hole indicating a reflection from a tilted surface (Figure A.5). An even better method is to use a pellicle beam splitter inserted before aperture A_1, such that the back-reflected light returning through A_1 is visible without disturbance from the illumination light (Figure A.6). It is paramount not to use any other type of beam splitter, as only the minuscule thickness of the pellicle beam splitter ensures that the optical axis defined by the laser will not be altered by this method.

When two lenses with a common focal plane are inserted into the beam path, shifting either of the lenses will alter the direction of the emerging beam. Thus, it is possible that the beam after the second lens points to the correct direction, although the beam is not centered correctly on the first lens. This could lead to suboptimal performance of the lens system.

Therefore, optical systems need to be aligned by inserting one lens at a time and keeping the already aligned lenses fixed.

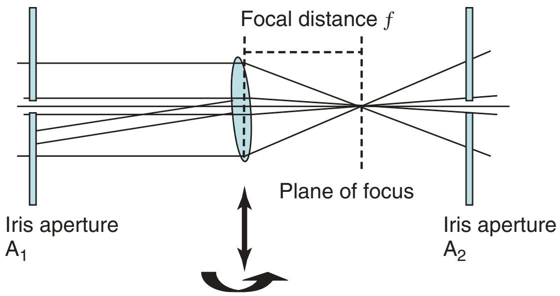

Figure A.5 Image demonstrating the use of iris apertures and back reflections from optical surfaces to adjust the position and tilt of optical elements.

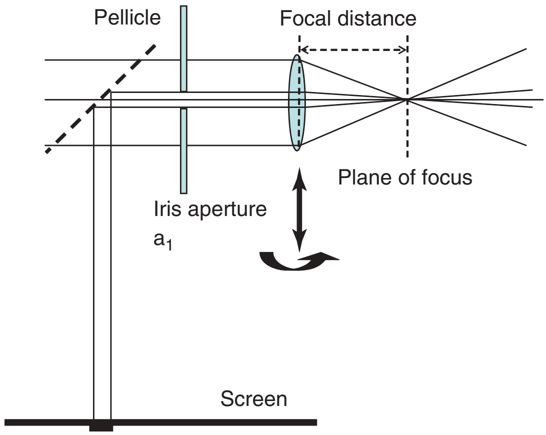

Figure A.6 Image demonstrating the use of a very thin membrane mirror (pellicle) and a pinhole to precisely fine-adjust the tilt of an optical element.

A.6 How to Find the Focal Plane of a Lens

It is often required to determine the focal distance of a lens. When this problem arises, the first thing one has to know is whether one is dealing with a single lens or with a lens system. With a single lens, one can, to a good approximation, assume the reference plane (the plane at which the rays of ray diagrams can be assumed to be bend), to lie inside this lens. When having determined the position of the focal plane (see below), the focal distance is then approximately given by the distance from the middle of the lens to this plane. Flipping the lens around should yield the same result. With lens systems (such as tube lenses of some microscope manufacturers), the situation can be much more complicated. These systems can have two different reference planes, one for each input from either side, which can lie far outside the actual lens system itself. The focal distances need to be measured from these reference planes. Here, we consider only the situation of a single lens.

The easiest method for determining the focal plane of a lens is to look at the image of an object placed at a great distance. If there is a window in the room, the objects in the sky, such as clouds, can be assumed to be at infinite distance compared to the lens focal distance. An image of the clouds will, therefore, be formed at the focal plane of the lens. For a first estimate, one can also use the lamp on the ceiling for this purpose, but only if the focal length of the lens is relatively short (e.g., smaller than 20 cm).

For lenses with very short focal length, it can be difficult to determine the plane of focus in this manner as the image may appear too small to be observed. However, when using a wide plane wave for illumination (i.e., a laser expanded to the full lens diameter using a beam expansion telescope), there is a useful trick to

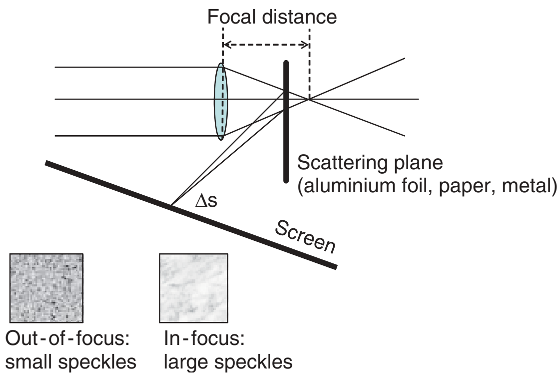

Figure A.7 Reflection at a rough surface to find the precise focus of a lens. The focal point can be determined by looking at the size of the speckles generated by the reflection off the rough surface. The better the focus, the larger the speckles.

determine the focal distance precisely (Figure A.7). If a piece of matte surface (such as the matte side of a tin foil) is mounted and moved along Z to find the focus, a strong speckle pattern is observed in the back-reflected light. One has to be very careful when working with lasers because the reflection off a matte surface can be dangerous. Thus, the lasers have to be at intensity ranges safe for the human eye. This speckle pattern stems from the interference of the waves scattered back by various heights of the surface with its microroughness. The size of these speckles is critically dependent on the optical path difference Δs (see Figure A.7) between different scattering positions on the matte plane, as shown in Figure A.7. The size of the speckles depends on the area that is illuminated on the surface; a small illuminated area will generate large speckles, whereas a larger illuminated area will generate smaller speckles. The position with the largest speckles in the diffuse reflected light therefore very accurately yields the correct focus position (method courtesy of Mats G. L. Gustafsson). This method can also be useful when focusing through the pinhole in a beam-expanding telescope.

A.7
How to Focus to the Back Focal Plane of an Objective Lens

In many setups (e.g., total internal reflection fluorescence (TIRF) or structured illumination), one needs to focus a laser beam to the back focal plane (BFP) of an objective lens. Strictly speaking, this should be called the front focal plane of the objective when the laser is used for the illumination of the sample. However, with the term *back focal plane*, which is usually used, one refers to the imaging path where the light is emitted by the sample. There is a simple trick to achieve this focusing to the BFP: when focusing to this point, a parallel beam spanning the field of view will leave the objective lens (Figure A.8). When pointing to the ceiling of

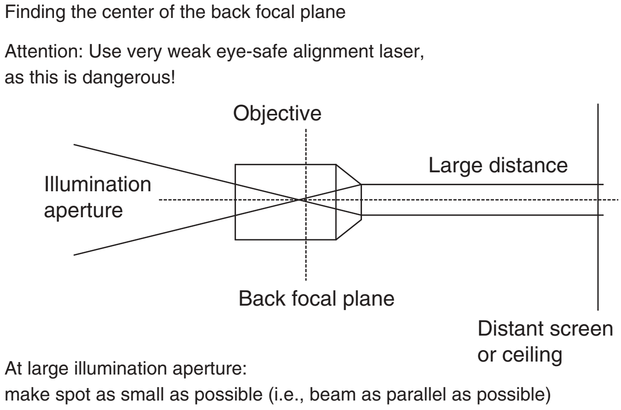

Figure A.8 Focusing a laser beam to the back focal plane of an objective lens generates a parallel beam of light after the objective. At a larger distance (e.g., meters), the smallest spot generated when altering the focal point indicates focusing onto the back focal plane (as seen in detection) of the objective. For laser safety reasons, extreme care has to be taken with this method. An eye-safe laser should be used and laser eye protection must be worn.

the room (in an inverse microscope), the beam should still be small. Minimizing the size of the beam leaving the objective at a larger distance, such as the ceiling of the room, allows determining whether the focus was adjusted correctly to the BFP. One has to be *extremely careful with this type of adjustment for laser safety reasons* as the laser beam remains parallel and small, leaving the objective that can easily point into any direction of space. Therefore, this procedure should only be used with lasers in the intensity range deemed safe for the human eye. For this adjustment, it is also necessary to focus the beam onto the BFP under a relatively large solid angle that is, not to use a small aperture defining the optical axis but to use the full aperture of the optical illumination system. If one only focuses a ~1 mm laser beam in this way, the field of view in the sample will be very small and thus the spot on the ceiling (inverted microscope) will be very big because of diffraction.

To adjust the beam to the precise center of the BFP, one has to ensure that the optical axis is marked at a far distant point with a pen with no objective being in place. Then the objective is inserted and the position of the focusing lens adjusted until the smallest possible spot is visible at the same position.